Role of Angiotensin Subtype 2 Receptor in Neointima Formation After Vascular Injury

Philip Janiak, Aline Pillon, Jean-Francois Prost, and Jean-Paul Vilaine

The role of angiotensin receptor subtypes 1 and 2 was assessed on neointima formation after injury in rat carotid artery. The effects of angiotensin converting enzyme inhibition by perindopril (3 mg · kg⁻¹ · day⁻¹ p.o.) and selective blockade of angiotensin subtype 1 receptors by DuP 753 (5 and 30 mg · kg⁻¹ · day⁻¹ p.o.) were compared on proliferative response to balloon injury. In rats treated 6 days before and for 14 days after injury, perindopril significantly reduced (−76%, p<0.01) myointimal hyperplasia. In contrast, DuP 753 at 5 mg · kg⁻¹ · day⁻¹ did not modify the hyperplastic response to balloon catheterization. Only at 30 mg · kg⁻¹ · day⁻¹ was DuP 753 able to reduce neointima formation (−47%, p<0.05). This dose was equipotent to perindopril on the renina-angiotensin system as assessed by the pressor response to angiotensin II and angiotensin I. Therefore, blockade of subtype 1 receptors was a less effective means of suppression of myointimal growth than angiotensin converting enzyme inhibition, suggesting that another angiotensin receptor subtype or converting enzyme substrates are involved in this process. For the determination of whether angiotensin subtype 2 receptors were implicated, the specific subtype 2 receptor antagonist CGP 42112A (1 mg · kg⁻¹ · day⁻¹) was continuously infused perivascularly for 14 days in the vicinity of the injured carotid artery. CGP 42112A was as effective in preventing neointima formation as perindopril (−73%, p<0.01, versus −76%, p<0.01, respectively). Our findings demonstrate that angiotensin subtype 2 receptors play a major role in myointimal formation after arterial injury and that this abnormal vascular response is associated with an increase in subtype 2 receptor activity. (Hypertension 1992;20:737–745)

KEY WORDS • angiotensin converting enzyme inhibitors • angiotensin II • receptors, angiotensin • angioptasia, transluminal • hyperplasia
specific antagonists such as CGP 42112A and have been found in uterus, kidney, adrenal glands, heart, arteries, and the brain, but their physiological roles remain unclear. Studies have shown that in the vascular system of the adult rat, the predominant Ang II receptor is the AT1 subtype with low density of AT1 subtype. However, during development, the ratio of AT1 to AT2 receptors in the vasculature is reversed.

The purpose of this study was to investigate the role of AT1 and AT2 angiotensin receptor subtypes in the proliferative response to vascular injury. Perindopril, a potent and long-acting ACE inhibitor, was used to inhibit ACE, and DuP 753 and CGP 42112A were used to selectively block AT1 and AT2 receptors, respectively.

Methods

All procedures were carried out in accordance with the guidelines of the French Ministry of Agriculture for the use and care of laboratory animals.

Cardiovascular Studies

To compare the effects of ACE inhibition versus the blockade of AT1 receptors on myointimal formation, it was first essential to determine the equipotency between perindopril and DuP 753 on the RAS. The first series of experiments was designed to select equally effective doses of perindopril and DuP 753 on the RAS after a 20-day chronic treatment. The duration of this treatment was the same as that subsequently used for the histomorphometric studies. Male Wistar rats weighing 400-450 g (Charles Rivers Laboratories, Saint-Aubin-les-Elboeuf, France) received either perindopril (3 mg kg\(^{-1}\) day\(^{-1}\) p.o.), DuP 753 (5 mg \(\text{kg}^{-1}\) \(\text{day}^{-1}\) p.o.), or their respective vehicles (1 ml \(\text{kg}^{-1}\) day\(^{-1}\) p.o.) for 20 days. On day 19, rats were anesthetized by intraperitoneal injection of a mixture of ketamine (100 mg kg\(^{-1}\)), and acepromazine (5 mg kg\(^{-1}\)), and the femoral artery and jugular vein were catheterized for arterial pressure measurement and drug administration, respectively. Both catheters were filled with heparinized saline (50 mg ml\(^{-1}\)) and were tunneled subcutaneously to exit at the back of the neck. Twenty-four hours after the last administration (day 21), mean arterial pressure (MAP) and heart rate (HR) were monitored in conscious, freely moving rats. Pulse pressure and MAP were displayed on a model ES1000 recorder (Gould Inc., Ballainvilliers, France). HR was determined by a Gould Biotech cardiotachometer providing the derivative function of arterial pulse pressure. ACE and AT1 receptor activities in the different groups were assessed by the pressor effects produced by intravenous injection of angiotensin I (Ang 1) (300 ng kg\(^{-1}\)) and Ang II (100 ng kg\(^{-1}\)), respectively.

To evaluate the activity level of the RAS at the time of balloon injury in rats chronically treated with perindopril and DuP 753, we performed the following protocol. After 4 days of oral treatment with either perindopril (3 mg \(\text{kg}^{-1}\) \(\text{day}^{-1}\)), DuP 753 (5 and 30 mg \(\text{kg}^{-1}\) \(\text{day}^{-1}\)), or their respective vehicles (1 ml \(\text{kg}^{-1}\) \(\text{day}^{-1}\)), male Wistar rats were anesthetized, and arterial and venous catheters were implanted as described above. Oral drug treatments were continued for the following 2 days. On day 7, MAP and HR were monitored, and the oral potency and duration of ACE inhibition and AT1 receptor blockade after perindopril and DuP 753 treatment, respectively, were examined as follows. After a 1-hour stabilization period, Ang I (300 ng kg\(^{-1}\)) or Ang II (100 ng kg\(^{-1}\)) was injected intravenously before and after oral administration of either perindopril (3 mg kg\(^{-1}\)) or DuP 753 (5 and 30 mg kg\(^{-1}\)). The effectiveness of the inhibition was examined for 6 hours and at 24 hours after administration.

Histomorphometric Studies

The first series of experiments was designed to compare the effects of ACE inhibition versus the blockade of AT1 receptors on neointima formation after arterial injury. For this study, six groups were used. Male Wistar rats weighing 400-450 g (Charles Rivers) were treated for 6 days before the endothelial denudation with either perindopril (3 mg \(\text{kg}^{-1}\) \(\text{day}^{-1}\) p.o.), DuP 753 (5 and 30 mg \(\text{kg}^{-1}\) \(\text{day}^{-1}\) p.o.), or their respective vehicles (1 ml \(\text{kg}^{-1}\) \(\text{day}^{-1}\) p.o.). These long-term treatments were continued for 14 days after injury. Endothelial injury was performed as follows in anesthetized rats (ketamine, 100 mg kg\(^{-1}\) i.p. and acepromazine, 5 mg kg\(^{-1}\) i.p.). A 2F balloon embolectomy catheter (Baxter, Mau- repas, France) was introduced through the external branch of the left carotid artery into the aortic arch. The intimal injury was performed by slowly pulling out the inflated balloon as described by Clowes et al. The procedure was repeated three times. The second series of experiments was performed to determine whether AT1 receptors were involved in the myointimal thickening. In a first experiment, rats received continuous subcutaneous infusion of the specific AT1 receptor antagonist CGP 42112A (1 mg \(\text{kg}^{-1}\) \(\text{day}^{-1}\)) or its vehicle via osmotic minipumps (models 2ML2, 2ML1, Alzet, Palo Alto, Calif.) for 20 days. As recommended by the manufacturer, the reservoir of the minipump was filled with the solution and incubated in a saline solution at room temperature for 24 hours to assure that the infusion rate was constant. After 6 days of subcutaneous infusion, balloon catheterization was performed as reported above. In a second experiment, rats with no drug pretreatment underwent balloon injury of the common carotid artery. At the end of the ballooning procedure (<2 minutes), a Silastic cuff connected by a catheter to an osmotic minipump (Alzet model 2ML2) was placed and secured around the injured artery to deliver a perivascular infusion of either CGP 42112A (1 mg \(\text{kg}^{-1}\) \(\text{day}^{-1}\)) or its vehicle.

Fourteen days after endothelial denudation (day 14), all rats were anesthetized by intraperitoneal injection of pentobarbital (60 mg kg\(^{-1}\)). Evans blue (0.5 ml of 0.5% saline solution) was administered intravenously 20 minutes before animals were killed to evaluate the level of endothelial regeneration. The carotid arteries were perfused under a pressure of 90 mm Hg with 10% formaldehyde for 20 minutes and then carefully dissected and left in the same fixative until processed further. The midportion of each carotid artery was used for histological studies, because this region did not show any evidence of endothelial regeneration (positive staining after Evans blue injection). The portion was divided in four segments (2.5 mm length) and embedded in paraffin. Cross sections (5 μm) were paraffinized, deparaffinized, and stained with orcein. The distance between two cross sections was 50 μm. Medial and intimal cross-sectional areas were measured with a Biocom computerized image analysis system (HISTO software, Biocom, Les...
Angiotensin Receptors and Intimal Hyperplasia

TABLE 1. Effects of 20-Day Treatment With Perindopril or DuP 753 on Mean Arterial Pressure and Heart Rate in Conscious Rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Vehicle (n=7)</th>
<th>Perindopril (n=7)</th>
<th>Vehicle (n=7)</th>
<th>DuP 753 (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>112±3</td>
<td>108±3</td>
<td>96±3*</td>
<td></td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>322±12</td>
<td>354±22*</td>
<td>328±9</td>
<td>336±9*</td>
</tr>
</tbody>
</table>

*b p<0.01; t not significant.

Data were analyzed by two-way analysis of variance with replication. Values are mean±SEM.

Results

Cardiovascular Studies

The first series of experiments was performed to evaluate the level of RAS activity after 20 days of chronic treatment with perindopril (3 mg · kg⁻¹ · day⁻¹) and DuP 753 (5 mg · kg⁻¹ · day⁻¹). Twenty-four hours after the last administration of the 20-day treatment (equivalent to day 14 in the histomorphometric studies), rats chronically receiving perindopril or DuP 753 showed a significantly lower MAP than their respective control groups. The blood pressure-lowering effects of perindopril and DuP 753 were not significantly different, and basal HR was not changed by either of the two treatments (Table 1). The pressor response produced by intravenous injection of Ang I (300 ng · kg⁻¹) and Ang II (100 ng · kg⁻¹) was used to determine the efficacy of perindopril on ACE activity and DuP 753 on the blockade of AT₁ receptors, respectively. In vehicle-treated groups, intravenous injection of Ang I (300 ng · kg⁻¹) produced a pressor response and a reflex bradycardia, which were similar in magnitude to the cardiovascular response mediated by intravenous injection of Ang II (100 ng · kg⁻¹) (Figure 1). On day 21, the pressor responses to Ang I and Ang II were reduced to a similar level by long-term treatment with perindopril (3 mg · kg⁻¹ · day⁻¹) and DuP 753 (5 mg · kg⁻¹ · day⁻¹), respectively.

The second set of experiments was carried out to determine whether perindopril (3 mg · kg⁻¹ · day⁻¹) and DuP 753 (5 and 30 mg · kg⁻¹ · day⁻¹) inhibited the RAS at the time of balloon catheterization (day 7) to the same extent and with the same time course. Twenty-four hours after the last administration of the six-day treatment, none of the drugs significantly modified MAP and HR (Table 2). In the perindopril-treated and the two DuP 753–treated groups, the pressor responses to intravenous injection of Ang I and Ang II, respectively, were significantly and similarly reduced compared with their responses in their respective control groups (p<0.01) (Figure 2). Subsequent administration of perindopril (3 mg · kg⁻¹) and DuP 753 (30 mg · kg⁻¹) almost completely abolished the pressor action of Ang I and Ang II, respectively, for at least 6 hours (p<0.01).

FIGURE 1. Bar graphs show effects of 20-day treatment with perindopril (3 mg · kg⁻¹ p.o., n=7) or DuP 753 (5 mg · kg⁻¹ p.o., n=8) on pressor response to intravenous injection of angiotensin I (ANG I) (300 ng · kg⁻¹) or angiotensin II (ANG II) (100 ng · kg⁻¹) in conscious rats. Twenty-four hours after last drug administration (day 21), pressor responses to ANG I and ANG II were reduced to a similar level by chronic treatment with perindopril and DuP 753, respectively. ΔMAP, change in mean arterial pressure; ΔHR, change in heart rate. Data were analyzed by two-way analysis of variance with replication. Values are mean±SEM. **p<0.01.
TABLE 2. Effects of 6-Day Treatment With Perindopril or DuP 753 on Mean Arterial Pressure and Heart Rate in Conscious Rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Vehicle (1 ml kg⁻¹) (n=7)</th>
<th>Perindopril (3 mg kg⁻¹) (n=6)</th>
<th>Vehicle (1 ml kg⁻¹) (n=5)</th>
<th>DuP 753 (5 mg kg⁻¹) (n=6)</th>
<th>DuP 753 (30 mg kg⁻¹) (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean arterial pressure</td>
<td>111±4</td>
<td>104±3</td>
<td>114±3</td>
<td>115±3</td>
<td>99±7</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>365±9</td>
<td>382±15</td>
<td>381±10</td>
<td>377±15</td>
<td>363±15</td>
</tr>
</tbody>
</table>

bpm, Beats per minute. Rats were treated for 6 days with either perindopril (3 mg kg⁻¹ day⁻¹ p.o.), DuP 753 (5 and 30 mg kg⁻¹ day⁻¹ p.o.), or their respective vehicles (1 ml kg⁻¹ day⁻¹ p.o.). Measurements were performed 24 hours after the last administration. Data were analyzed by one-way analysis of variance. Values are mean±SEM.

However, rats receiving the low dose of DuP 753 (5 mg kg⁻¹) did not show the same profile. Despite the fact that the pressor response to Ang II remained similarly decreased 24 hours after the 6-day treatment, new administration of DuP 753 at 5 mg kg⁻¹ was less effective in reducing the increase in MAP elicited by Ang II than at 30 mg kg⁻¹ (Figure 2, bottom panel).

**Histomorphometric Studies**

The results of the histomorphometric studies are illustrated in Figures 3 and 4. Fourteen days after endothelial injury, a massive proliferation was observed in the intima of the vehicle-treated groups. Hyperplasia was not statistically different among all vehicle-treated groups. The neointima area and the intima/media ratio were significantly reduced by perindopril treatment (−75% and −76%, respectively; p<0.01). In contrast, DuP 753 treatment at 5 mg kg⁻¹ day⁻¹ did not alter neointimal hyperplasia. In this group, the neointima area and the intima/media ratio were close to those observed in the corresponding vehicle-treated group. Only long-term treatment with high doses of DuP 753 (30 mg kg⁻¹ day⁻¹) significantly attenuated myointimal thickening (−47%). Chronic subcutaneous infusion of CGP 42112A (1 mg kg⁻¹ day⁻¹) did not modify the proliferative response to vascular injury. However, when the same dose of CGP 42112A was constantly infused in the perivascular vicinity of the injured artery, the neointima area and the intima/media ratio were significantly suppressed (78% and 73%, respectively; p<0.01).

**Discussion**

Ang II receptors, ACE, and angiotensinogen messenger RNA have been identified in the vascular wall, and several lines of evidence have supported the role of a local RAS in the pathogenesis of neointima formation induced by vascular injury. In rats treated 6 days before injury and continuously for the subsequent 14 days, Ace inhibition by perindopril (3 mg kg⁻¹ day⁻¹) significantly reduced (−76%) myointimal hyperplasia induced by endothelial denudation. These findings were identical to those described for cilazapril (−77%), where the same model of neointima hyperplasia and protocol design were used. In comparison with other ACE inhibitors, perindopril appears to be one of the most potent in suppressing myointimal proliferation since cilazapril and captopril showed similar activity but at much higher doses (10 and 100 mg kg⁻¹, respectively). In this respect, Plissonnier et al have also reported the potency of perindopril on another model of myointimal growth induced by aortic allograft. In this...
model, low doses of perindopril (1 mg · kg⁻¹ · p.o., twice daily) significantly decreased intimal thickening.

Because inhibition of ACE was associated with a reduction in neointima formation, decreased Ang II concentration has been suggested to be the cause for this antihyperplastic effect. Chronic infusion of Ang II stimulated DNA synthesis, enhanced the neointima formation, and abolished the protective action of ACE inhibitors on intimal hyperplasia when administered concomitantly.6 These data strongly supported the role of Ang II in this aberrant vascular response and excluded the contribution of other putative peptides whose accumulation might also result from ACE inhibition. DuP 753 reduced neointima formation induced by balloon injury only when high doses were used. Indeed, at 5 mg · kg⁻¹ · day⁻¹, neointimal growth was not affected, but at 30 mg · kg⁻¹ · day⁻¹, the proliferative response was reduced by 47% (p < 0.05). This latter dose of DuP 753 showed the same efficacy and time course of RAS inhibition as perindopril at 3 mg · kg⁻¹ · day⁻¹, whereas 5 mg · kg⁻¹ · day⁻¹ of DuP 753 was not as effective in blocking the RAS at the time of balloon injury. Therefore, a high dose of DuP 753 was more suitable for the histomorphometric studies, where the purpose was to compare the effects on myointimal growth of ACE inhibition versus blockade of AT₁ receptors. These data confirm those reported recently, in which high doses of DuP 753 (20 mg · kg⁻¹ · day⁻¹ p.o. and 15 mg · kg⁻¹ · day⁻¹ i.v.) were indicated to reduce myointimal thickening (~45% and ~48%, respectively).6,18 whereas DuP 753 given intravenously at 5 mg · kg⁻¹ · day⁻¹ was not effective despite its hypotensive effect similar to an intravenous dose of 15 mg · kg⁻¹ · day⁻¹.

Thus, the effects of ACE inhibition and AT₁ blockade on myointimal proliferation differ markedly despite a similar level of RAS inhibition. If stimulation of AT₂ receptors contributed preferentially to the neointima formation, selective blockade of these receptors should have suppressed the hyperplastic response to the same extent as after ACE inhibition. However, the maximal protective activity found with ACE inhibitors (cilazapril, captopril, perindopril) is always superior to that reported for DuP 753 (approximately ~80% versus ~50%, respectively).6,16 regardless of the dose and protocol used. Nonetheless, ACE does not cleave only Ang I but also bradykinin, substance P, and possibly other peptides. Therefore, the accumulation of some of these peptides due to ACE inhibition might be responsible for the effects on vascular proliferation. Thus, inhibition of converting enzyme is known to potentiate the physiological responses of bradykinin. A recent study claimed that part of the antiproliferative effect of ACE inhibition could be attributed to a reduction in the breakdown of bradykinin.19 Chronic blockade of bradykinin receptors with Hoechst 140 blunted but did not abolish the protective effect of ACE inhibition, suggesting that blockade of Ang II formation and an increase in endogenous kinins would be the mechanisms by which ACE inhibitors suppress the vascular response to injury.19 Bradykinin could mediate its antiproliferative effect, if any, by stimulating the endothelial release of prostacyclin and endothelium-derived relaxing factor. However, after vascular injury, the endothelium is totally removed and thus the major source of prostacyclin and endothelium-derived relaxing factor. This finding leaves the debate open and needs to be confirmed by other studies, particularly because bradykinin has been documented to act as a growth factor and to possess mitogenic activity in vitro.20,22 Therefore, increased bradykinin concentration would have been expected to stimulate the vascular response to injury.

The major finding of our study is that AT₂ receptors play an important role in the neointima formation induced by vascular injury in the rat. Chronic blockade of AT₂ receptors by perivascular infusion of CGP 42112A (1 mg · kg⁻¹ · day⁻¹ i.v.) markedly reduced the vascular response to balloon catheterization (~73%, p < 0.01). Only CGP 42112A infusion in the perivascular vicinity of the injured carotid artery was able to match the effect of ACE inhibitors on myointimal formation. When the same dose of CGP 42112A was given subcutaneously, the proliferative response remained unchanged despite a 6-day pretreatment. The inefficacy of CGP 42112A when administered under these conditions could have been explained by heat degradation that would occur by leaving this compound in solution in minipumps at 37°C for 2 weeks. However, this is not the case, because the high-performance liquid chromatographic profile of CGP 42112A from the solution remaining in minipumps after 2 weeks of infusion was identical to the original one (degradation <5%; unpublished observations). The difference of efficacy between these two routes of administration could be due, for instance, to a difference in drug delivery or effective half-life of this compound, but we cannot rule out other possibilities.

Quantitative autoradiographic studies have shown that, in the vascular system of the adult rat, the predominant Ang II receptor is the AT₁ subtype with low density of AT₂ (30%). However, the expression of AT₂ receptors is elevated during development, because the number of AT₂ receptors and the AT₁/AT₂ receptor ratio are much higher in fetal than in adult rat. This switch in the expression of AT₁ and AT₂ receptors suggested that AT₂ receptors may play a role in the vascular development. Similar change in the expression of AT₁ versus AT₂ has been observed in other tissues, such as the skin, where the expression of AT₂ receptors is enhanced during experimentally induced wound healing. The ability of a tissue to switch its proportion of Ang II receptors from AT₁ to AT₂ has also been reported in the myometrium during ovine pregnancy. Our results indicate not only that AT₂ receptors are involved in myointimal growth but that this pathological vascular response is associated with an increase in AT₂ receptor expression. Overall, the participation of Ang II to vascular response after injury involved both AT₁ and AT₂ receptor subtypes.

For many years, the role of Ang II in vascular growth has been limited to cell hypertrophy and facilitation of growth factor–induced proliferation. For instance, Geiserter et al. indicated that Ang II induced hypertrophy but not hyperplasia in cultured rat aortic SMCs with a contractile phenotype and stimulated protein synthesis. In contrast, Campbell-Boswell and Robertson reported that Ang II was able to induce proliferation of SMCs isolated from human aorta. Recently, a direct proliferative effect of Ang II has been demonstrated in cultured SMCs of hypertensive rats and in human fetal mesangial cells. This mitogenic effect in mesangial cells was substantially reduced in adults. Nonetheless,
there is evidence that the growth response to Ang II may vary as a function of the phenotypic state of SMCs. Based on their ability to proliferate and to synthesize extracellular proteins, and on their inability to contract, cells from the neointima and cultured cells originating from normal media have been considered to belong to the same synthetic phenotype. However, there is some evidence that neointimal cells represent a unique sub-

**FIGURE 3.** Photomicrographs show representative histological cross sections of rat carotid artery 14 days after balloon injury. Panel A: Uninjured rat carotid artery; panel B: injured rat carotid artery treated with vehicle; panel C: injured rat carotid artery treated with perindopril (3 mg·kg⁻¹·day⁻¹ p.o.); panel D: injured rat carotid artery treated with DuP 753 (30 mg·kg⁻¹·day⁻¹ p.o.); panel E: injured rat carotid artery treated with CGP 42112A (1 mg·kg⁻¹·day⁻¹; perivascular infusion). Orcein stain. Note strong reduction of neointimal area in carotid artery from rat treated with perindopril and with CGP 42112A. Bar, 100 μm.

**FIGURE 4.** Bar graph shows effects of perindopril (3 mg·kg⁻¹·day⁻¹ p.o., n=7), DuP 753 (5 mg·kg⁻¹·day⁻¹ p.o. [n=10] and 30 mg·kg⁻¹·day⁻¹ p.o. [n=7]), and CGP 42112A (1 mg·kg⁻¹·day⁻¹ s.c. [n=11] and 1 mg·kg⁻¹·day⁻¹ p.v. [n=8]) on myointimal formation 14 days after endothelial denudation performed by balloon catheterization in rat carotid artery. Intima/media ratio was significantly and similarly reduced by perindopril given orally and CGP 42112A infused perivascularly in the vicinity of injured carotid artery (~76% and ~73%, p<0.01, respectively). In contrast, only DuP 753 at 30 mg·kg⁻¹·day⁻¹ significantly altered vascular response (~47%, p<0.05) but to a lesser extent than perindopril and perivascular CGP 42112A. Data were analyzed by unpaired Student's t test. Values are mean±SEM. *p<0.05; **p<0.01.
type of SMCs distinct from cultured cells originating from normal media. In culture, these cells secrete large amounts of platelet-derived growth factor–like molecules, show platelet-derived growth factor–independent growth, transcribe the platelet-derived growth factor B chain, synthetize fetal proteins, and have a distinct morphology, epithelioid in shape, which is similar to endothelial cells. SMCs isolated from newborn animals share these characteristics, as opposed to cultured medial SMCs from adult animals, which do not. From these observations arises the possibility that neonontal SMCs could express a fetal phenotype, which would result from the amplification of a subpopulation of medial SMCs. Because AT2 is widely expressed and is the predominant angiotensin subtype during embryonic life, it is possible that the neonontal cells originating from this subpopulation with fetal properties would express mainly AT2 receptors. This hypothesis could explain why cultured aortic SMCs originating from normal media show only AT1 receptors. Our theory is also consistent with the selectivity of action of Ang II on SMCs of injured arteries. In balloon catheter–injured arteries, Ang II stimulates DNA synthesis and growth more markedly in the neointima than in the media. This histological selectivity in the proliferative response to Ang II could be due to a predominant expression of AT2 receptors in the neointima of injured arteries.

Intimal thickening in response to arterial injury results from the combination of three major events, including migration of SMCs from the media to the intima, proliferation of intimal SMCs, and production of extracellular matrix. The mechanism by which the blockade of AT1 receptors inhibits the development of this vascular lesion could be due to either inhibition of SMC migration from the media to the intima or a decrease of proliferation of intimal SMCs since DuP 753 did not modify the cell density of the neointima. In fact, according to Prescott et al, DuP 753 inhibited both SMC migration and proliferation, because DNA synthesis in medial SMCs was decreased 24–48 hours after injury, and the number of SMCs at day 4 after injury was reduced. At day 4, intimal SMCs are supposed to represent the number of SMCs that have migrated from the media to the intima. Therefore, it would also be interesting to study the mechanism by which AT2 antagonist suppresses the development of this vascular lesion, to investigate whether the combination of AT1 and AT2 receptor blockade prevents the vascular response more effectively than ACE inhibition alone, and how AT1 and AT2 receptor stimulation could interplay.

The signal-transduction pathway of the AT2 receptor remains poorly understood. In tissue expressing AT2 receptors exclusively, it has been shown that this angiotensin receptor subtype is not coupled to G proteins; does not affect inositol trisphosphate production, intracellular calcium mobilization, or adenylate cyclase activity; and is not a receptor mediating internalization of agonist. However, recent reports indicate that, in neonatal neuronal cells, AT2 stimulation reduced cyclic GMP, probably through a mechanism involving calcium influx and the activation of cyclic GMP phosphodiesterase, and in astrocytes, it increased prostaglandin synthesis. Interestingly, AT2 receptors are localized in highly vascularized organs, such as uterus, kidney, brain, heart, and adrenal glands, and recently, a novel physiological role has been reported for AT2 receptors that could be linked to these observations. Indeed, Le Noble et al demonstrated that Ang II stimulates angiogenesis in the chorioallantoic membrane model in a dose–dependent manner. This effect was blocked by CGP 42112A, suggesting that AT2 receptors mediated this angiogenic activity.

In conclusion, our findings demonstrated for the first time the participation of AT2 receptors in the processes of excessive repair that lead to lesion formation in response to vascular injury. It remains to be established whether the beneficial effects of AT2 receptor blockade holds in other species, for example, in swine or primate, in which ACE inhibitors have been reported to be ineffective in suppressing neointima formation. This is an important issue because the recent results of the MERCATOR restenosis trials show that the ACE inhibitor cilazapril does not reduce restenosis after angioplasty, despite a profound effect on the intimal proliferative response to balloon injury in the rat. These negative results in humans correlate well with those observed in swine and primate and support the necessity of studies on other species before the therapeutic relevance of the blockade of AT2 receptor in restenosis after balloon angioplasty is considered.

Acknowledgment

We gratefully acknowledge the expert statistical assistance of Corinne Thomas-Haimez.

References


38. Le Noble FAC, Schruers N, Van Straaten HWM, Slaaf DW, Smits JFM, Struyker Boudier HAJ: Angiotensin II induced angiogenesis is not mediated through the AT1 receptor. (abstract) *FASEB J* 1992;6:A937.


Role of angiotensin subtype 2 receptor in neointima formation after vascular injury.

P Janiak, A Pillon, J F Prost and J P Vilaine

Hypertension. 1992;20:737-745
doi: 10.1161/01.HYP.20.6.737

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1992 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/20/6/737

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/